

NATIONAL CHILDREN'S STUDY PRECONCEPTION CORE HYPOTHESIS—ASSISTED REPRODUCTIVE TECHNOLOGY

I. Proposed Hypothesis

Children whose conceptions were aided by infertility treatments such as the assisted reproductive technologies (ARTs) are at increased risk of fetal growth restriction, birth defects and developmental disabilities in comparison to children whose conceptions were unassisted by such treatments.

II. Workgroup

Fertility and Early Pregnancy Working Group (Possible Collaboration with Exposure to Chemical Agents; Growth)

III. Contacts

Germaine Buck & Robert Chapin

IV. Public Health Significance

Prevalence: Intrauterine growth restriction (IUGR) is typically measured as a function of birth weight relative to estimated gestation. The prevalence of IUGR is estimated to be 30 to 250 per 1000 births depending on the definition and reference population used.¹ The prevalence of birth defects is estimated to be approximately 30 to 40 per 1000 infants born, though in the United States, national data are not available and data from individual states vary according to the coding systems that are used and the manner in which cases are ascertained.^{2,3} The prevalence rates for specific birth defects vary widely; for example, 1999 prevalence estimates from the Metropolitan Atlanta Congenital Defect Program are 0.1 per 1000 liveborn infants for spina bifida, 1.7 per 1000 liveborn infants for clubfoot, and 3.7 per 1000 liveborn infants for hypospadias.⁴ Developmental disabilities are defined as a group of physical, cognitive, psychological, sensory, and speech impairments that begin anytime during development up to 18 years of age. The prevalence of developmental disabilities (all types) is estimated at 170 per 1000 U.S. children under 18 years of age.⁴ Severe developmental disabilities include mental retardation, cerebral palsy, hearing loss, vision impairment, and epilepsy. The prevalence of severe developmental disabilities is estimated at 20 per 1000 school-aged children. More specifically, the Metropolitan Atlanta Developmental Disabilities Surveillance Program provides population-based prevalence estimates of selected developmental disabilities; these are 9.7 per 1000 children ages 3-10 for mental retardation, 2.8 per 1000 for cerebral palsy, 1.1 per 1000 for hearing loss, 0.9 per 1000 for vision impairment, and 3 per 1000 for autism spectrum disorders.⁴

In the United States and worldwide, the use of assisted reproductive technologies to overcome infertility is increasing rapidly. Assisted reproductive technologies (ARTs) are defined as those infertility treatments in which both oocytes and sperm are handled in the laboratory; these include in vitro fertilization-transcervical embryo transfer, gamete and zygote intrafallopian transfer (gametes or zygotes transferred into fallopian tubes rather than uterus), frozen embryo transfer, and donor embryo transfer. In 1999, the most recent year for which U.S. population-based data are available, more than 86,000 ART procedures were performed, resulting in more than 30,000 live-born infants.⁵ These infants represent an estimated 0.8 percent of the total infants born in the United States in 1999. This proportion is expected to continue to rise, largely due to improved accessibility and successful treatments. Further, ART treatments represent only a fraction of the infertility treatments currently used. Results from a national survey suggest that in 1995, treatment with ovulation stimulation medications without ARTs was 30 times more frequent than the use of ARTs, and artificial (or assisted) insemination was 10 times more frequent (separate statistics for ARTs and assisted insemination were obtained through a personal communication with A. Chandra, NCHS).⁶ Thus, the proportion of infants conceived using various infertility treatments, is ostensibly orders of magnitude higher than the 0.8 percent

estimated for ARTs alone. As states increasingly ensure medical coverage for fertility treatment, economic barriers will disappear and services will be widely available to all couples. Recent data suggest that more programs are beginning to offer ART services. Specifically, between 1996 and 1998 there was a 14 percent increase in programs participating in the U.S. ART Registry maintained by the CDC in collaboration with the Society for Assisted Reproductive Technology (SART).⁷ For these same years, there was a 26.5 percent increase in the total number of ART procedures reported. In sum, more and more couples with impaired fecundity or fertility are overcoming their underlying conditions and conceiving children with the assistance of medical technology. ART treatments are the most invasive and technologically advanced within the spectrum of infertility treatments currently available.

Another reason that children born with the assistance of ARTs are of special concern is that pregnancies achieved with the use of these technologies are more likely to result in multiple births. For example in 1998, 56% of U.S. infants born following the use of ARTs were multiples.⁷ Recent data suggest that ARTs alone accounts for just over 9 percent of twin (unpublished data, CDC) and more than 40 percent of the triplet and higher order births in the United States, with other infertility treatments likely accounting for a sizable proportion of the remainder.⁸

Morbidity: IUGR has been shown to be associated with a number of outcomes, ranging from neonatal morbidity, to developmental disabilities and chronic conditions during childhood.^{1,9-13} Major birth defects are associated with increased infant and childhood mortality and long-term disability.² Developmental disabilities are linked with various medical conditions throughout the lifespan. Infants conceived with ART therapies are reported to be at increased risk for low birth weight, particularly at term, and birth defects (see below). There is also emerging evidence that ART infants may be at increased risk for developmental disabilities. However, studies to date for all three outcomes have been primarily retrospective and of varying methodological quality.

Quality of Life: Infants born of diminished birth size and or with a major birth defect or severe developmental disability are at increased risk for a multitude of health, psychosocial, and education adversities.^{1,2,4,9-12}

Mortality: Perinatal and infant mortality rates are inversely related to decrements in fetal growth and birth size.^{14,15} Birth defects are the leading cause of infant mortality in the United States.¹⁴

Cost: Children with birth defects and/or those born too small contribute disproportionately to infant and pediatric health care costs.^{2,16} For example, over 25 percent of pediatric hospital admissions are estimated to occur among children with birth defects.² Children with developmental disabilities require a host of special education services, medical services and supportive care. In the US special education costs are estimated at \$36 billion annually.⁴

Perceived Importance: IUGR holds clinical and public health significance because it is an important intermediate outcome in a wider spectrum of disorders. In the short-term, IUGR is associated with a host of neonatal complications and increased mortality.^{1,9,12-15} Additionally, both animal and human studies suggest IUGR may predispose an individual to longer-term outcomes such as developmental disabilities during childhood and chronic conditions affecting numerous tissues and organ systems (e.g., impaired glucose tolerance, abdominal obesity, decreased HDL cholesterol, increased systolic and diastolic blood pressure, cardiovascular mortality, altered lung function, renal complications, altered thyroid function) beginning in childhood and continuing into adulthood.^{1,9-12} Thus, IUGR may hold important clues for the fetal origins of adult disease.

Children born with congenital anomalies are at increased risk of morbidity and mortality during infancy and childhood. In addition, they often have numerous physical, developmental, and learning disabilities. Birth defects are estimated to be a major contributor to potential years of life lost in the United States.²

Developmental disabilities are lifelong conditions with substantial morbidity. The etiologies for most developmental disabilities are poorly understood. Scant epidemiologic data are available.

Multiples are at increased risk of preterm delivery, IUGR, birth defects, death and long-term morbidity including developmental disability.¹⁷⁻²¹ They should be adequately represented in the National Children's Study, given their biologic immaturity which may render them particularly susceptible to environmental agents.

Recently, there has been a lot of attention paid to the financial burdens faced by couples attempting to conceive using ARTs and other infertility treatments. In addition, and even more importantly, questions have been raised about the safety of ARTs for the children that are conceived, both singletons and multiples. A prospective study of the relation between ART and outcomes such as IUGR and birth defects would be an important step in addressing these concerns. Research on short and long-term health effects related to the treatments, the underlying conditions leading to infertility, or possible interactive effects between the two, has not kept pace with the rapid advances in technology.

V. Justification for a Large, Prospective, Longitudinal study

Although IUGR is a fairly prevalent outcome, a study of the longer-term health outcomes that are less prevalent than IUGR, will require a large sample of pregnancies and infants. Although studies to date have documented associations between IUGR and a plethora of long-term outcomes, a more exact understanding of the mechanisms underlying these associations is needed. While the hypothesis of early fetal programming is intriguing, prior studies that have attempted to address the issue have been criticized on a number of methodological shortcomings.¹⁰ Longitudinal studies beginning prior to conception are needed to disentangle the various etiologies and sub-types of IUGR, to monitor fetal growth from very early in pregnancy, to detect changes in fetal growth prospectively, and to track the long-term consequences. In addition, the prospective design will enable scientists to detect additional adverse outcomes, which have been unidentified to date including those that result in termination or fetal death.

A large prospective study is also the appropriate design to study birth defects. This is the only design that will allow for the identification of nearly all incident cases, including those that result in fetal death. Additionally, prospective follow-up of live-born infants will allow for complete case ascertainment, as a proportion of defects will not be identifiable at birth. Finally, the tracking of live-born infants will also allow for more careful assessment of the impact that various exposures (including infertility treatment) may have on the long-term health status of infants with various types of defects.

Because developmental disabilities are diagnosed throughout childhood, a prospective study design is needed to appropriately follow children and capture and track these outcomes as well.

Prospective data collection is also needed to obtain complete and standardized exposure data. Such data can be roughly divided as: 1) exposures during the pre-conceptional period that may be related to both a couple's underlying infertility and the subsequent risks for IUGR and birth defects among the children conceived with ARTs; 2) the underlying pathophysiology(ies) responsible for a couple's infertility (which may directly impact the pregnancy); and 3) the specification of the infertility treatment(s) used. The National Children's Study is uniquely qualified to address these issues.

Pre-conceptional exposures of interest include environmental toxicants, nutritional factors, and individual behavioral factors, such as cigarette smoking and alcohol use. Retrospective collection of these data will be limited by poor maternal and paternal recall and will result in a lack of biological specimens collected at critical periods. Prospective interviews, diaries and biological sampling will greatly improve the accuracy and breadth of these exposure data.

Maternal recall of both infertility diagnoses and infertility treatment is poor, despite the level of invasiveness of many treatment modalities (C. Ghosh, Doctoral Dissertation, 2000; personal communication Dr. Mary Croughan.) Retrospective medical record review may also be limited because women in the United States commonly seek treatment in multiple clinics and the clinics themselves commonly close or reorganize, which impairs the ability to obtain records (personal communications, various members of SART.) Also, medical evaluation and laboratory testing for infertility diagnoses is highly variable across clinics (unpublished data, CDC). Prospective data collection will allow for consistent and comprehensive infertility evaluations. This is of primary importance because a key methodological concern with previous studies that examined this hypothesis or related hypotheses, is that it is not possible to completely distinguish effects caused by the infertility treatment from effects caused by the underlying infertility (see below.) Finally, prospective data collection will also allow for complete and accurate information on a number of specific treatment factors. These include use of highly invasive techniques such as intracytoplasmic sperm injection (including prospective semen parameters on the male partner or sperm donor), assisted hatching, pre-implantation genetic diagnoses, specifics of culture media (including the use of co-culture or blastocyst culture), and early and consistently timed ultrasound data on the number of gestational sacs and fetal hearts (currently there is wide variation in whether and how such data are recorded across clinics.)

VI. Scientific Merit

ART is recognized as an important contributor to the U.S. low birth weight rate because of the known association between the use of ARTs and multiple births^{5, 7, 8} and between multiple births and low birth weight.²² Additionally, studies have suggested that low birth weight rates are increased among singleton infants conceived with ARTs as compared with naturally conceived infants or population-based rates.²³⁻³¹ These previous studies were limited in their assessment of specific treatment-related effects. Moreover, questions remain about whether the reported low birth weight risk for singletons conceived with ARTs is a direct effect of the procedure or reflects some other factor related to the underlying infertility of the couples who conceive using these procedures. Studies examining these hypotheses have reported conflicting findings.³¹⁻³⁶ One recent study based on the population-based registry of ART-conceived infants in the United States found that singleton infants who were conceived using ARTs and were born at term had a low birth weight risk that was over two times higher than expected, based on comparison with the general U.S. population of singleton births.³¹ This increased risk persisted after adjusting for maternal age and parity. Additionally, the higher than expected risk remained even when the sample was limited to infants conceived with eggs from apparently fertile women (i.e. donor eggs) and infants carried by women who were unlikely to have an underlying uterine disease (i.e. women seeking ARTs because their male partner had an infertility diagnosis and women serving as a gestational surrogate for the ART patient). These subgroup findings suggest that the increased risk for term low birth weight may be related to the ART procedure itself, rather than the underlying infertility. However, this registry dataset does not contain detailed clinical information on patients' underlying medical conditions. A study that closely follows ART patients and their pregnancies prospectively is needed to more definitively address this important question. To date, few studies have specifically evaluated low birth weight due to IUGR separately from low birth weight due to early delivery, and no study has monitored fetal growth changes prospectively.

With respect to birth defects, equivocal results exist regarding the association between ARTs (including intracytoplasmic sperm injection) and birth defects in the offspring.^{23-27, 29-30, 37-41} Prior studies have suffered from various methodological problems including low statistical power, particularly to assess individual defects separately, and differential case ascertainment and coding schemes for infants conceived using ARTs and infants conceived naturally. Nearly all studies relied on retrospective registry data. Two recent studies have shown an increased risk for various birth defects among ART-conceived infants.⁴⁰⁻⁴¹ These studies are particularly noteworthy because they demonstrated elevated risks even among singleton infants. Specific malformations implicated were neural tube defects, alimentary tract atresia, omphalocele and hypospadias in one study⁴⁰ and cardiovascular, urogenital, chromosomal, and musculoskeletal defects in the other study.⁴¹ However, small sample sizes limited the assessments of specific organ systems. Both studies were based on retrospective registry data and were unable to separate effects caused by specific treatment exposures from

effects that may have been related to a couple's underlying infertility. Again, a large, well-designed prospective study is needed to address this question.

The study of longer term outcomes such as developmental disabilities among ART children has been hampered by inadequate sample sizes and lost to follow-up. A recent study, reported an increased risk for developmental delay and cerebral palsy among children conceived with ART.⁴² These effects remained elevated when analyses were limited to singleton births; however, the study suffered from a number of methodological drawbacks including a lack of statistical power to adequately assess subgroup findings and thus a large prospective study would greatly advance this important research question.

This proposed core hypothesis focuses on 3 important outcomes – IUGR, birth defects, and developmental disabilities. These have been implicated as potential adverse outcomes associated with ARTs. It is, however, important to note that there is a paucity of evidence-based findings on the safety of ARTs in terms of the general long-term health status of the children. In addition to neurodevelopmental disorders, concerns have been raised about chronic conditions during childhood and continuing into adulthood and impaired reproductive function and fecundity among this F1 generation. Inclusion of this hypothesis as a core National Children's Study hypothesis will also ensure a study design with the capacity to identify a number of health outcomes among this growing population of children.

VII. Potential for Innovative Research

Specimen and data collection during pre- and peri-conceptional period will facilitate development of sensitive molecular markers in the human for this temporal window of sensitivity that results in substantial loss of life. Estimates of peri-conceptional pregnancy loss in the human far surpasses any other species that has been studied, ranging as high as 62 percent.⁴³ In addition, collection of this sensitive and timed exposure data may have long-term implications for understanding the fetal origins of adult disease.

VIII. Feasibility

Critical Period: Many exposures of interest act prior to the clinical recognition of pregnancy. In addition, many exposures might adversely impact the gametes prior to conception.

Sampling Needs: There are several key sampling issues to consider when sampling ART pregnancies:

- There is a variable lag time between when couples begin trying to conceive and when they begin infertility treatment. In order to capture this population, a sampling strategy that incorporates selected recruitment at infertility treatment centers will be required.
- Couples seeking ARTs are already undergoing intensive and time-consuming medical testing and treatments. It is anticipated that data collection could be structured to coincide with their regular visits to the infertility clinic.
- Not all ART treatments will be successful. The average pregnancy rate of ART treatments is 31 percent and the average livebirth rate is 25%, with a high degree of variability according to the woman's age.⁴ Couples who are not successful with the first treatment often undergo multiple ART procedures (in 1999, nearly 50 percent of patients undergoing an ART procedure reported having a previous ART treatment.) As would be expected, success rates are slightly higher for couples on their first attempt. Thus, in designing the optimal approach for sampling this group, it will be important to consider ART patient selection factors such as age, duration of infertility, previous ART attempts, and perhaps some general information about the infertility diagnosis or clinical presentation. For example, the pregnancy rate for women under 35 years of age with no previous ART attempts who present with normal levels of follicle stimulating hormone and estradiol is estimated at 41% (unpublished data, CDC). This then might represent a subgroup of ART patients to target.

- The study design should ensure that the sample selected is appropriate to study the effects of ART on various outcomes for singleton births separately. Because such a high proportion of ART births are multiple, this must be factored into sample size estimations.
- Certain sub-types of ART, might be of particular interest. At present, there is a great deal of concern about the safety of ICSI, above and beyond ART alone. Thus, one potential option is to select two ART samples – e.g. in vitro fertilization with ICSI and without ICSI. (Note: ART covers a somewhat heterogeneous group of treatments and thus in addition to targeting certain ART patients for recruitment, it will also be highly desirable to restrict recruitment to certain types of ART procedures – for example, one might restrict to in vitro fertilization [both with and without ICSI] among patients who used embryos created using their own eggs that were freshly fertilized [i.e. exclude donor eggs and frozen-thawed embryos, and instead focus on the most commonly used ART type – IVF +/- ICSI with fresh non-donor eggs and embryos])

Estimates of the sample size necessary to study various outcomes among singleton infants conceived with ART are presented in Table 1. As can be seen these sample size estimates were derived with conservative assumptions of the minimum risk ratio – 1.5 for each of the general outcomes: IUGR, preterm delivery, birth defects, serious developmental disabilities, and mild-serious developmental disabilities; and 2.0 for specific individual or related groups of birth defects or developmental disabilities. Studies to date have suggested that risk ratios may indeed be higher. Other assumptions include the following:

- For every ART singleton there will be a minimum of 4 non-ART singletons in the comparison group.
- 35% of ART deliveries will be to multiple births
- 15% of ART pregnancies will be lost prior to birth (a conservative estimate if targeted recruitment of younger women is used – unpublished data CDC).

Sample sizes vary widely given the range of prevalence rates for the outcome measures. A minimum sample of 4,335 ART pregnancies is needed to study each of the general outcomes with sufficient power to assess ART singletons separately. To study specific birth defects and developmental disabilities the necessary sample size increases to as much as 8,798 ART pregnancies depending on the prevalence of the defect or disability. (Of note: although certain individual defects and disabilities are even less prevalent than the 0.3% minimum prevalence presented in the table, it is probably not feasible to study these as individual defects/disabilities; however, such very rare defects might be grouped with other defects thought to be etiologically similar to increase statistical power to satisfactory levels.)

As mentioned previously, one bonus of considering ART infants separately would be to additionally capture a large sample of multiple births. If 4,335 ART pregnancies are sampled, an estimated 1,290 sets of multiple births would be included in addition to the 2,395 singleton births; likewise if 8,798 ART pregnancies are sampled, 2,617 multiple birth sets would be included.

Contact: Couples will need to be contacted at varying intervals, depending on the nature of the measurements. It is anticipated that contact would most often occur at the infertility clinic during regularly scheduled visits for evaluation and treatment.

Nature of Measurement: Various methods of data collection could be utilized, ranging from diaries and interviews to urine and blood sample collection.

Burden on the Participant and Family: The data and specimen collection would be time intensive, but participants would receive a large amount of valuable information. Couples trying to conceive using infertility treatments such as ARTs have expressed the need for more data on the safety of these procedures (personal communication, RESOLVE.)

Ethical Considerations: Privacy and confidentiality issues are paramount concerns for sampling and data collection procedures. The so-called sensitive data (e.g., ART) are entitled to the same confidentiality issues

as so-called non-sensitive data. The protection of privacy for all study participants regardless of recruitment source is a must. As with all data on environmental exposures, there may be insufficient evidence for interpreting risk. Risk communication will be essential for ensuring open communication with study participants.

REFERENCES

1. Pryor J. The identification and long term effects of fetal growth restriction. *British J Obstet Gynaecol.* 1996;103:1116-22.
2. Lynberg MC, Edmonds LD. State use of birth defects surveillance. In: Wilcox LS, Marks JS editors. *From Data to Action: CDC's Public Health Surveillance for Women, Infants, and Children*. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services. 1994 pp. 217--26.
3. Congenital malformations surveillance report: A report from the National Birth Defects Prevention Network. *Teratology.* 2000;61:1-160.
4. Centers for Disease Control and Prevention. National Center on Birth Defects and Developmental Disabilities. Website. <http://www.cdc.gov/ncbddd/default.htm>.
5. Centers for Disease Control and Prevention (CDC) American Society for Reproductive Medicine, Society for Assisted Reproductive Technology, RESOLVE. 1999 Assisted reproductive technology success rates: national summary and fertility clinic reports, Atlanta, GA: CDC;2001.
6. Abma J, Chandra A et al. Fertility, family planning and women's health: New data from the 1995 National Survey of Family Growth. *NCHS Vital Health STat* 23 (19) 1997.
7. Centers for Disease Control and Prevention. Use of assisted reproductive technology — United States, 1996 and 1998. *Morbidity and Mortality Weekly Report* 2002;51:97-101.
8. Centers for Disease Control and Prevention. Contribution of assisted reproductive technology and ovulation-inducing drugs to triplet and higher-order multiple births -- United States, 1980–1997. *Morbidity and Mortality Weekly Report* 2000;49:535-8.
9. Institute of Medicine. *Preventing Low Birthweight*. Washington, DC: National Academy Press, 1985.
10. Joseph KS, Kramer MS. Review of the evidence of fetal and early childhood antecedents of adult chronic disease. *Epidemiologic Reviews*. 1996;18:158-74.
11. Godfrey KM, Barker DJP. Fetal nutrition and adult disease. *Am J Clin Nutr.* 2000; 71:1344s-52s.
12. Alberman E. Low birthweight and prematurity. In: Pless IB, ed. *The epidemiology of childhood disorders*. New York: Oxford University Press; 1994:49-65.
13. Hack M, Flannery DJ, Schluchter M, Cartar L, Borawski E, Klein N. Outcomes in young adulthood for very-low-birth-weight-infants. *N Engl J Med*, 2002; 346:149-157.
14. National Center for Health Statistics. Infant mortality statistics from the 1998 period linked birth/infant death data set. *National Vital Statistics Report*; 48, no. 12. Hyattsville, Maryland: US Department of Health and Human Services, CDC, National Center for Health Statistics, 2000.
15. Rees JM, Lederman SA, Kiely JL. Birth weight associated with lowest neonatal mortality: infants of adolescent and adult mothers. *Pediatrics* 1996;98:1161-6.

16. Sandhu B, Stevenson RC, Cooke RW, Pharoah PO. Cost of neonatal intensive care for very-low-birthweight infants. *Lancet* 1986; 8481: 600-3.
17. Kiely JL. What is the population-based risk of preterm birth among twins and other multiples? *Clin Obstet Gynecol* 1998;41:3-11.
18. Guyer B, Martin JA, MacDorman MF, Anderson RN, Strobino DM. Annual summary of vital statistics--1996. *Pediatrics* 1997;100:905-918.
19. Pharoah POD, Cooke T. Cerebral palsy and multiple births. *Arch Dis Child Fetal Neonatal Ed* 1996;75:F174-F177.
20. Gardner MO, Goldenberg RL, Cliver SP, Tucker JM, Nelson KG, Copper RL. The origin and outcome of preterm twin pregnancies. *Obstet Gynecol* 1995;85:553-557.
21. Spellacy WN, Handler A, Ferre CD. A case-control study of 1253 twin pregnancies from a 1982-1987 perinatal data base. *Obstet Gynecol* 1990;75:168-171.
22. Martin JA, Park MM. Trends in twin and triplet births: 1980-97. Hyattsville, MD: National Center for Health Statistics;1999: National vital statistics reports; Vol. 47 No. 24.
23. Westergaard HB, Tranberg Johansen AM, Erb K, Anderson AN. Danish national in-vitro fertilization registry 1994 and 1995: a controlled study of births, malformations and cytogenetic findings. *Hum Reprod* 1999;14:1896-1902.
24. Dhont M, De Sutter P, Ruysink G, Martens G, Bekaert A. Perinatal outcome of pregnancies after assisted reproduction: a case-control study. *Am J Obstet Gynecol* 1999;181:688-95.
25. Verlaenen H, Cammu H, Derde MP, Amy JJ. Singleton pregnancy after in vitro fertilization expectations and outcome. *Obstet Gynecol* 1995;86:906-10.
26. Bergh T, Ericson A, Hillensjo T, Nygren KG, Wennerholm UB. Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: a retrospective cohort study. *Lancet* 1999;354:1579-85.
27. FIVNAT (French In Vitro National). Pregnancies and births resulting from in vitro fertilization: French national registry, analysis of data 1986 to 1990. *Fertil Steril* 1995;64:746-56.
28. Gissler M, Silvero MM, Hemminki E. In-vitro fertilization pregnancies and perinatal health in Finland 1991-1993. *Hum Reprod* 1995;10:1856-61.
29. Friedler S, Mashiach S, Laufer N. Births in Israel resulting from in-vitro fertilization/embryo transfer, 1982-1989: National Registry of the Israeli Association for Fertility Research. *Hum Reprod* 1992;7:1159-63.
30. MRC Working Party on Children Conceived by In Vitro Fertilisation. Births in Great Britain resulting from assisted conception, 1978-87. *BMJ* 1990;300:1229-33.
31. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;346:731-737.

32. Sundstrom I, Ildgruben A, Hogberg U. Treatment-related and treatment-independent deliveries among infertile couples, a long-term follow-up. *Acta Obstet Gynecol Scand* 1997;76:238-43.
33. Olivennes F, Rufat P, Andre B, Pourade A, Quiros MC, Frydman R. The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. *Hum Reprod* 1993;8:1297-1300.
34. Henriksen TB, Baird DD, Olsen J, Hedegaard M, Secher NJ, Wilcox AJ. Time to pregnancy and preterm delivery. *Obstet Gynecol* 1997;89:594-9.
35. Williams MA, Goldman MB, Mittendorf R, Monson RR. Subfertility and the risk of low birth weight. *Fertil Steril* 1991;56:668-71.
36. McElrath TF, Wise PH. Fertility therapy and the risk of very low birth weight. *Obstet Gynecol* 1997;90:600-5.
37. Bonduelle M, Legein J, Buysse A, et al. Prospective follow-up study of 423 children born after intracytoplasmic sperm injection. *Hum Reprod* 1996;11:1558-1564.
38. Buysse WA, Ban Assche E, Kevroey P, Van Steirteghem, Liebaers I. Prospective follow-up study of 1987 children born after intracytoplasmic sperm injection (ICSI). Abstract: Treatment of Infertility: The New Frontiers, Boca raton, Florida, 1998.
39. Wennerholm UB, Bergh C, Hamberger L, Lundin K, Nilsson L, Wikland M, Kallen B. Incidence of congenital malformations in children born after ICSI. *Hum Reprod* 2000;15:944-948.
40. Ericson A and Kallen B. Congenital Malformations in Infants Born After IVF: a Population-Based Study. *Hum Reprod* 2001;16:504-509.
41. Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med* 2002;346:725-730.
42. Stromberg B, Dahlquist G, Ericson A, Finnstrom O, Koster M, Stjernqvist KK. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet* 2002;359:461-465.
43. Edmonds KD, Lindsay KS, Miller JF, Williamson E, Wood PJ. Early embryonic mortality in women. *Fertil Steril* 1982; 38: 447-53.

Table 1. Sample size estimates to study various outcomes among singleton infants – National Children’s Study preconception Core Hypothesis – Assisted Reproductive Technology

1	2	3	4	5	6
Outcome of Interest	Prevalence in general population	Minimum expected increase in ART singleton population compared to general population	What sample size of singleton births is needed in ART group (exposed)? Assumptions: At least 4:1 ratio non-ART (non-exposed) to ART infants $\alpha=0.05$ (two-sided) $\beta=0.2$	What is the total sample size of ART live birth deliveries needed to obtain # singletons in Column 4? Assumptions: 65% of deliveries will be singletons; 35% will be multiple birth sets	What is the total sample size of ART pregnancies needed to obtain # of deliveries in Column 5? Assumptions: 15% fetal loss rate
General Outcomes					
Intrauterine growth restriction (term infants)	5%	1.5 fold (7.5%)	925	1,423	1,674
Preterm delivery	10%	1.5 fold (15%)	434	668	809
Birth defects (all)	3%	1.5 fold (4.5%)	1,578	2,428	2,856
Serious developmental Disabilities (all)	2%	1.5 fold (3%)	2,395	3,685	4,335
Mild-serious developmental disability (all)	17%	1.5 fold (25.5%)	233	358	421
Specific Outcomes					
Specific birth defect or developmental disability	1.0%	2.0 fold (2%)	1,445	2,223	2,615
Specific birth defect or developmental disability	0.5%	2.0 fold (1%)	2,909	4,475	5,265
Specific birth defect or developmental disability	0.3%	2.0 fold (0.6%)	4,861	7,478	8,798